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Micropsalliota pseudoglobocystis, a new species from China

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Abstract — *Micropsalliota pseudoglobocystis* sp. nov. from China is introduced. The species is described and illustrated, and its phylogenetic placement is determined using molecular data. The new species is compared with the most similar taxa in the genus. This is the first report of the genus *Micropsalliota* from China.

Key words — *Agaricaceae*, taxonomy, phylogeny

Introduction

Micropsalliota Höhn. (*Agaricaceae*) was established by Höhnel (1914) and later emended by Pegler & Rayner (1969) and Heinemann (1976). *Micropsalliota* species are often encountered at the sides of forest trails in tropical areas (Zhao et al. 2010). *Micropsalliota* is similar to *Agaricus* L. as both genera have an annulus on the stipe, free gill attachment, and brown basidiospores. Phylogenetic analyses of ITS and LSU sequence data have established that *Micropsalliota* is monophyletic (Zhao et al. 2010). *Micropsalliota* can be distinguished from other similar genera with dark spore prints by its small to medium sized fruiting bodies, ellipsoid to cymbiform basidiospores with an apical thickening in the endosporium, often capitate or subcapitate cheilocystidia, and pileipellis hyphae encrusted with a pigment that turns olive to green in NH_4OH (Zhao et al. 2010).

Heinemann (1980, 1983, 1988, 1989), Heinemann & Little Flower (1983), and Heinemann & Leelavathy (1991) described about 40 species from the tropics of Africa, America, India, Indonesia, and Malaysia. Zhao et al. (2010) have since added eleven new taxa to this genus from northern Thailand. There are presently no reports of *Micropsalliota* species from China.

A survey of *Agaricaceae* has been carried out in southwestern China since 2012. Morphological and molecular studies have revealed some undescribed species, one of which is formally introduced in this paper as *Micropsalliota pseudoglobocystis*.

Materials & methods

Morphological examination

Specimens were collected from Tongbiguan National Natural Reserve located at Yingjiang County, Yunnan Province, China, on 19 July 2013. Photographs were taken in the field and specimens were wrapped in aluminum foil and kept separately in a box in order to avoid mixing or crushing. Macromorphological features were described and macrochemical reactions were made from fresh samples as soon as possible after returning from the field. Specimens were dried overnight in a food drier, sealed in plastic bags, and deposited in the herbarium of Southwest Forestry College, Kunming, China (SWFC) and Herbarium Mycologicum Academiae Sinicae, Beijing, China (HMAS).

Anatomical features (including the pileipellis, partial veil, basidiospores, basidia, and cystidia) were recorded from dry specimens following the protocols of Largent (1986). Dimensions were calculated from at least 20 measurements of anatomical features (spores, basidia and cystidia) and abbreviated as x = mean length by width ± SD; Q = quotient of basidiospore length and width; and Q_m = mean of Q-values ± SD.

Molecular experiment and phylogenetic analysis

DNA was extracted from the dried specimens using the E.Z.N.A. Forensic DNA Extraction Kit (D3591-01, Omega Bio-Tek). ITS regions and 5.8S rDNA were amplified using primers ITS4 and ITS5 according to Zhao et al. (2010) with some modifications. PCR products were sent to Shuoyang Biotechnology Company for sequencing.

Newly produced sequence and sequences downloaded from GenBank were aligned using BioEdit v. 7.1.3.0 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) and ClustalX 2.0 in default setting (Thomson et al. 1997). The alignments have been submitted to TreeBASE (submission ID 16500).

Maximum Parsimony (unweighted) analysis were performed using PAUP*4.0b10 (Swofford 2004). One thousand heuristic searches were conducted with random sequence addition, with tree bisection-reconnection (TBR) branch swapping and gaps treated as missing data. Parsimony bootstrap values were obtained from 1000 bootstrap replicates, with starting trees obtained via stepwise addition, random sequence addition, TBR branch swapping, and Max-trees set to 1,000,000.

MrModeltest 2.2 was used to determine the appropriate model of nucleotide substitution for this data for Maximum likelihood analysis and Bayesian inference (Nylander 2004). Maximum likelihood (ML) analysis was performed in PAUP*4.0b10

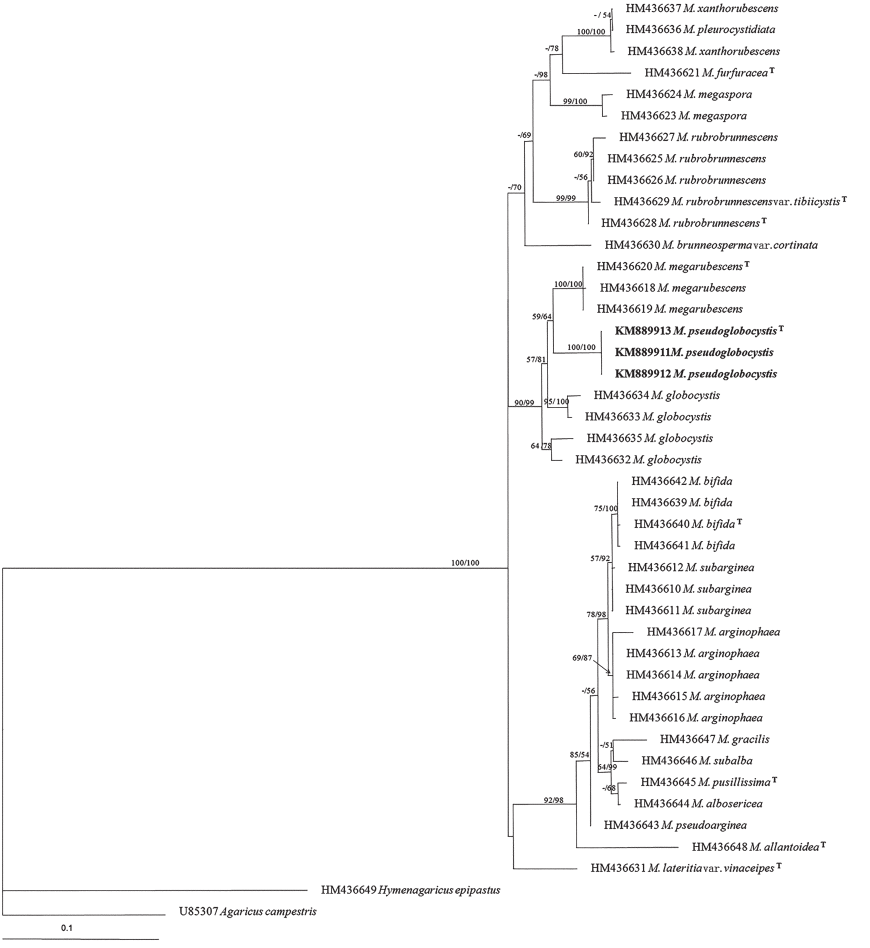


Figure 1 Phylogeny of *Micropsalliota* generated from Maximum Likelihood analysis of ITS sequences, rooted with *Agaricus campestris* and *Hymenagaricus epipastus.* Parsimony bootstrap support (BS) and Bayesian posterior probability (PP) values >50% are given at the internodes (BS/PP). "T" indicates a sequence from the type specimen. The new sequences produced from this research are in bold.

with a GTR+I+G model of nucleotide substitution, starting trees obtained via stepwise addition, random sequence addition and TBR branch swapping. Bayesian analysis was performed using Metropolis-coupled MCMC methods in MrBayes 3.1.2 with a GTR+I+G model and an inv-gamma distribution rate variation across sites. Chains were run for two parallel searches from random starting trees for 1 million generations

and trees were sampled every 100 generations. Those trees sampled prior to searches reaching a split deviation frequency value reaching 0.01 were discarded as burn-in (Huelsenbeck & Ronquist 2001; Huelsenbeck et al., 2001; Ronquist & Huelsenbeck 2003).

Results

The ITS dataset included sequences from 43 specimens representing 22 taxa of *Micropsalliota*, with *Agaricus campestris* and *Hymenagaricus epipastus* as the outgroup (Zhao et al. 2010). The dataset had an aligned length of 733 characters in the dataset, of which 122 characters were excluded from all analyses, 389 characters are constant, 87 variable characters are parsimony-uninformative, and 135 characters are parsimony-informative. Maximum parsimony analysis recovered one equally parsimonious tree (L = 497 steps, CI = 0.626, RI = 0.806, RC = 0.504, HI = 0.374). Maximum likelihood analysis produced a single topology (–lnL = 3155.4316; Fig. 1). Bayesian analysis resulted in a single topology with an average standard deviation of split frequencies = 0.009789. The ML and Bayesian topologies are nearly identical, differing only in the position of *M. lateritia* var. *vinaceipes*. The ML tree is shown in Fig. 1.

According to the tree, *Micropsalliota* species form a monophyletic group with strong support (100BS/100PP values). The three samples cluster together with strong support (100BS/100PP values), and form a clade sister to *M. megarubescens* and *M. globocystis*.

Taxonomy

Micropsalliota pseudoglobocystis Li Wei & R.L. Zhao, **sp. nov.** Fig. 2

MycoBank MB 810860

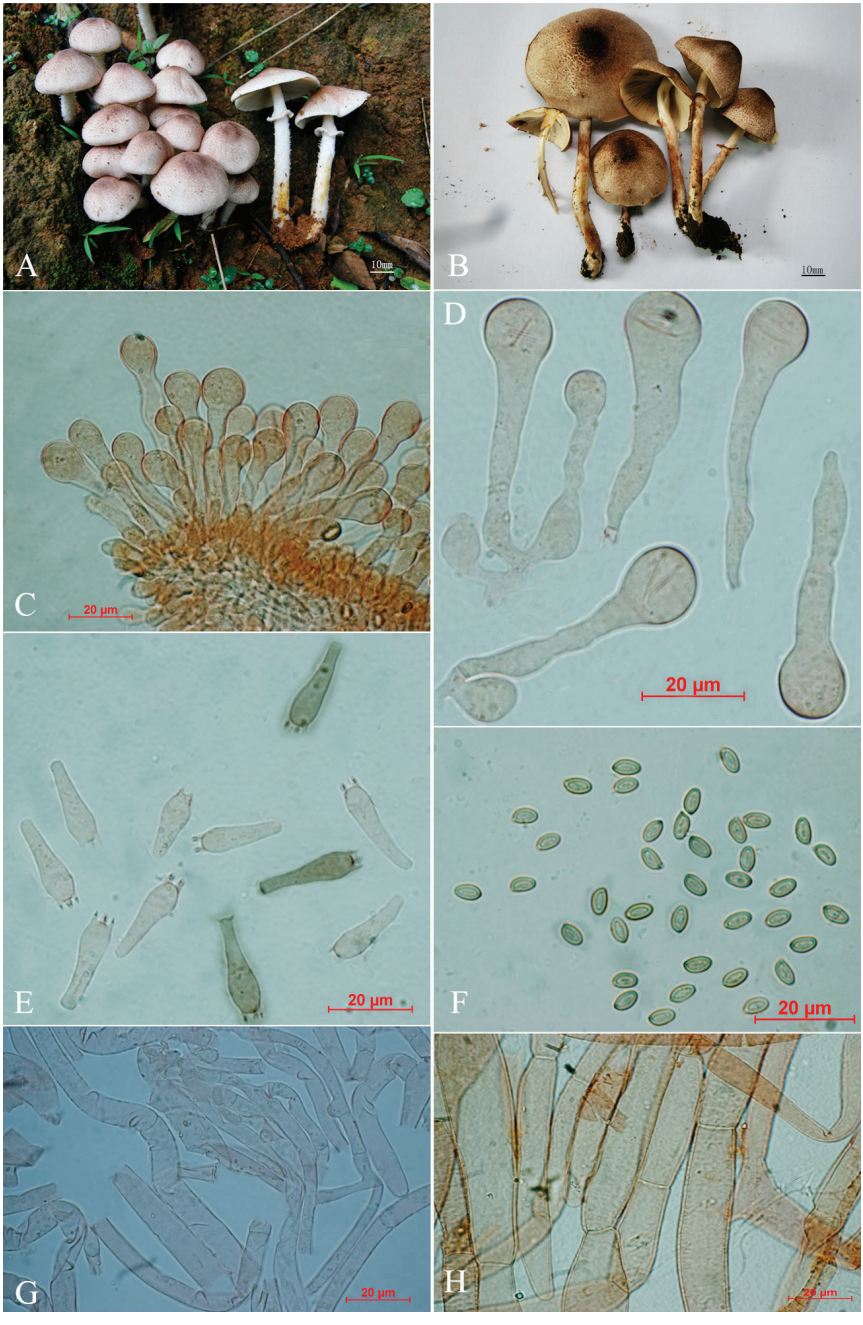
Differs from *Micropsalliota globocystis* by its smaller basidiospores and its stipe covered by heavily fibrillose scales.

Type: China, Yunnan Province, Yingjiang County, Tongbiguan National Natural Reserve, 19 July 2013, collected by Qing-Hua Yu ZRL201332 (**Holotype** HMAS; **isotype**, SWFC; GenBank, KM889913).

Etymology: the epithet refers to the morphological and phylogenetic similarity to *Micropsalliota globocystis*.

Pileus 25–35 mm diam., broadly and obtusely conical when young, then expanding to 45–55 mm in diam., convex and subumbonate; margin recurved, crenate, splitting with age, rarely uplifted; surface dry, covered with dense fibrillose-scales, recurved, reddish-brown against the whitish background.

Figure 2 *Micropsalliota pseudoglobocystis* (holotype): A, B. Macrocharacters and discoloring on cutting and bruising; C, D. Cheilocystidia; E. Basidia; F. Basidiospores; G. Annulus hyphae; H. Pileipellis hyphae.


A
10mm
B
10mm
C
20 μm
D
20 μm
E
20 μm
F
20 μm
G
20 μm
H
20 μm

Context 2 mm thick, white. Lamellae free, crowded, with 2–4 series of lamellulae, 2–4 mm broad, at first white, then grayish white, finally dull brown, edges even entire. Stipe 42–126 × 5–8(–10) mm, cylindrical, sometimes with basal rhizomorphs, hollow, surface above annulus glabrous to fibrillose, below annulus floccose, scaly, white. Annulus pendent, single, membranous, superior, edge entire, persistent, white, 4–6 mm broad, upside smooth, lower side heavily fibrillose. Odor of seaweed. Staining bright yellow then reddish-brown when bruised or in exposure.

Macrochemical reaction: KOH reaction strongly reddish brown, dark brown in stipe.

Basidiospores 4.5–6 × 2.5–3.2 µm [$x = 5.1 \pm 0.3 \times 2.8 \pm 0.2$, Q = 1.6–2.1, $Q_m = 1.8 \pm 0.13$, n = 20], ellipsoid, amygdaliform, with apical thickening (endosporium), no germ pore, brown. Basidia 15–20 × 5–7 µm, clavate, hyaline, 4-spored. Cheilocystidia 40–50 × 10–13 µm, irregularly cylindrical to subclavate with a subcapitate to capitates apex, and often present a long, narrow and flexuous neck, smooth, hyaline. Pleurocystidia absent. Pileipellis a cutis composed of hyphae 7–18 µm diam., constricted at the septa, incrusted, contains brown membranous pigments. Annulus composed of hyphae of 5–10 µm diam. smooth, hyaline and branched.

Habit: gregarious on the bank of a forest trail.

Additional material examined: **China**, **Yunnan Province**, Yingjiang County, Tongbiguan National Natural Reserve, 19 July 2013, collected by Qing-Hua Yu ZRL2013323 (SWFC; GenBank KM889912); ZRL2013335 (SWFC; GenBank KM889911).

Discussion

Other species of *Micropsalliota* with reddish-brown or purple brown scales on the pileus are *M. arginophaea* Heinem., *M. furfuracea* R.L. Zhao et al., *M. megaspora* R.L. Zhao et al., *M. pleurocystidiata* Heinem. & Little Flower, *M. xanthorubescens* Heinem., and *M. globocystis* Heinem. (Heinemann 1976, 1980; Zhao et al. 2010). All of these species differ from *M. pseudoglobocystis* morphologically, as well as phylogenetically: *M. arginophaea* does not discolor on bruising or cutting and has brown pileus scales; *M. furfuracea* has larger pileus scales; *M. megaspora* has larger basidiospores (6–7 × 3.5–4 µm); *M. pleurocystidiata* has pleurocystidia; *M. xanthorubescens* has medium-sized basidiomata (pileus 40–70 mm diam.); and *M. globocystis* has distinctly larger basidiospores (6–8 × 3.5–4.2 µm) and its stipe has a tomentose surface. Another phylogenetically closely related species is *M. megarubescens* R.L. Zhao et al., which differs by being slightly fibrillose on the disc of the pileus and glabrous elsewhere (Zhao et al. 2010).

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